

# Reverse phase HPLC of class I HLA eluted peptide ligands

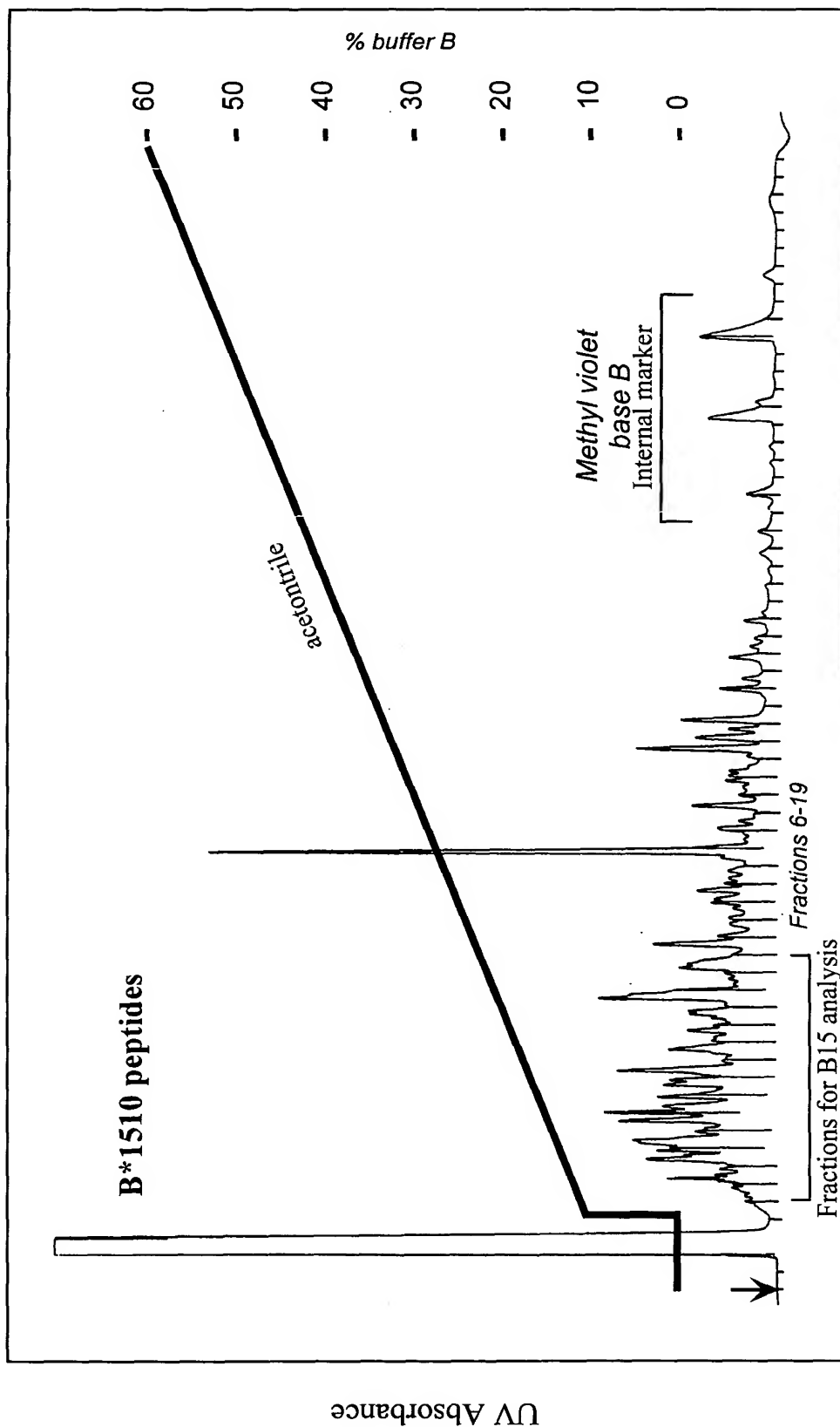


Fig. 1

Ion maps of peptides eluted from various B15 class I sHLA molecules. Mapping was accomplished with a nano-spray needle and an ESI mass spectrometer. The figure shows that the same ion peak is present in 3 of 4 B15 class I.

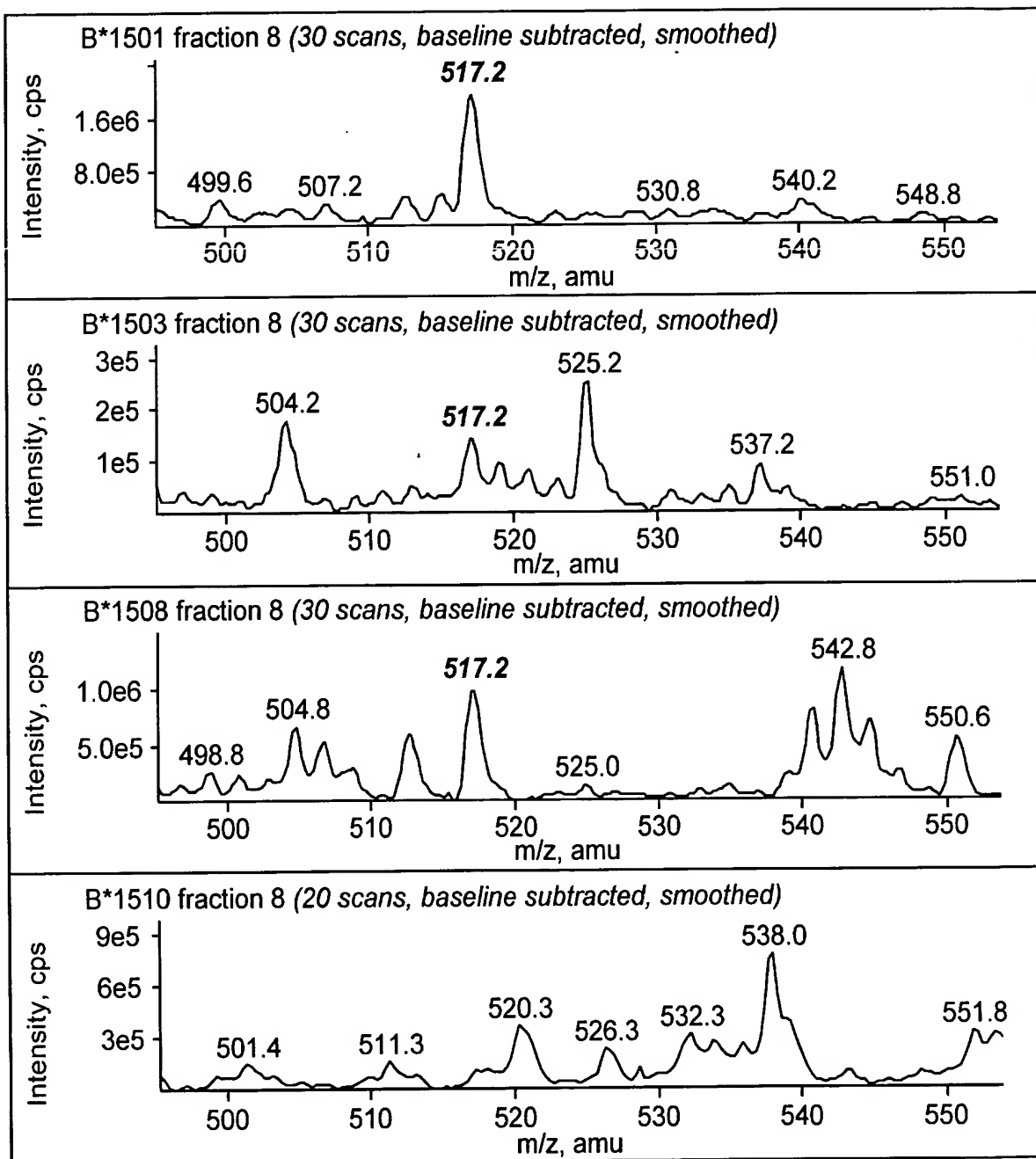


Fig. 2

209290"HEADSHOT

MS/MS fragmentation-sequencing of ion 517.2 from the various B15 class I sHLA molecules. This data was accomplished by completing a second nanospray of the peptides in fraction 8 from the HPLC. This demonstrates how ions can be MS ion mapped and subsequently MS/MS sequenced. There is sufficient peptide present to do multiple MS/MS fragmentation runs. There is also sufficient peptide present to facilitate a submotif on fraction 8 or further separation in the event that two peptides had mapped at 517.2 in the ion map.

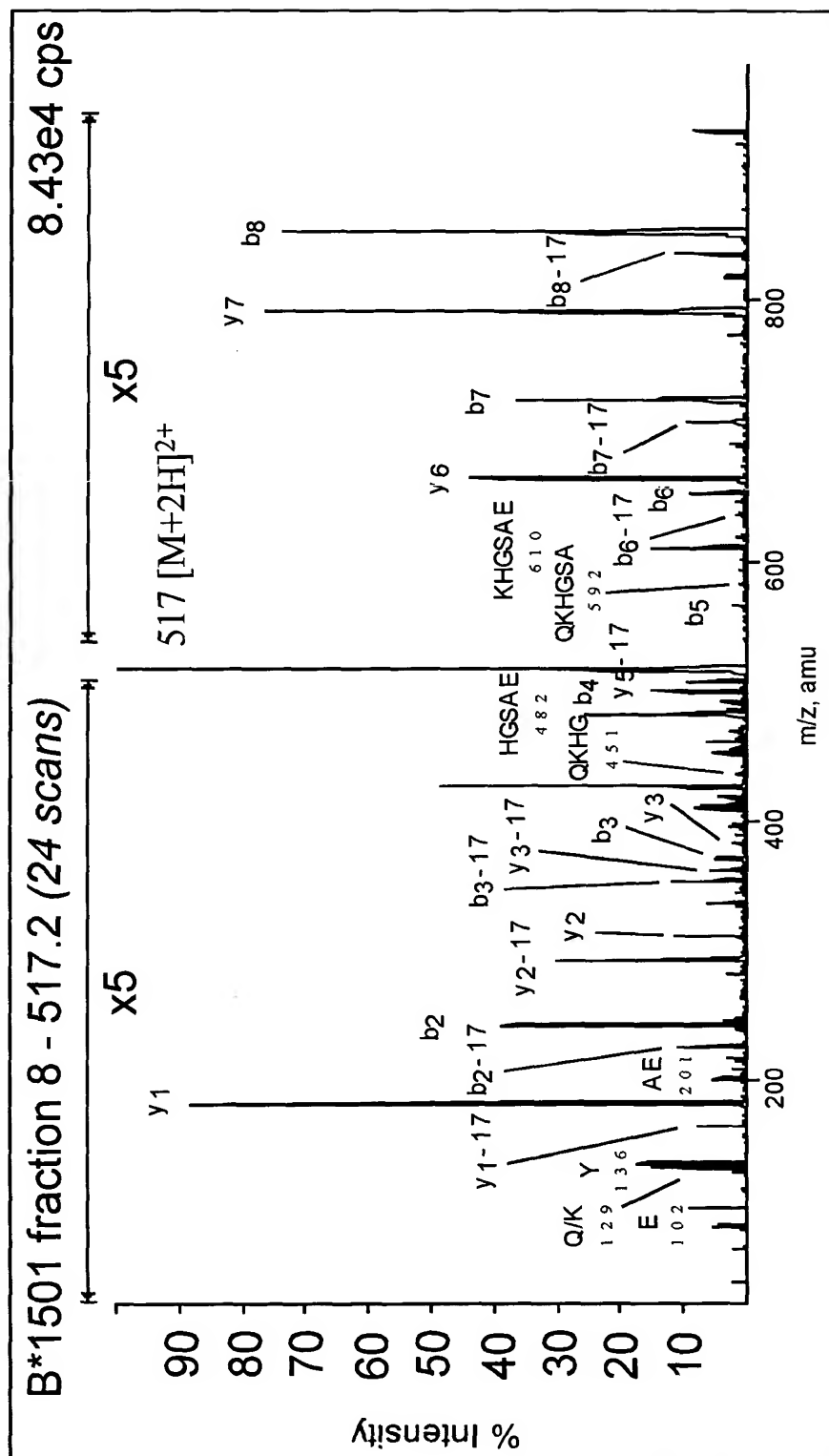


Fig. 3 1 of 3

Serial No. 10/082,034 Dkt. No. 6680.040  
Title: SOLUBLE HLA LIGAND DATABASE UTILIZING  
PREDICTIVE ALGORITHMS AND METHODS OF MAKING  
AND USING SAME  
Applicant: William Hildebrand Group No.: 2171  
Filed: 02/21/2002 Examiner: Unknown  
Atty: Douglas J. Sorocco Tel: (405) 478-5344

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2092910-HE02800F

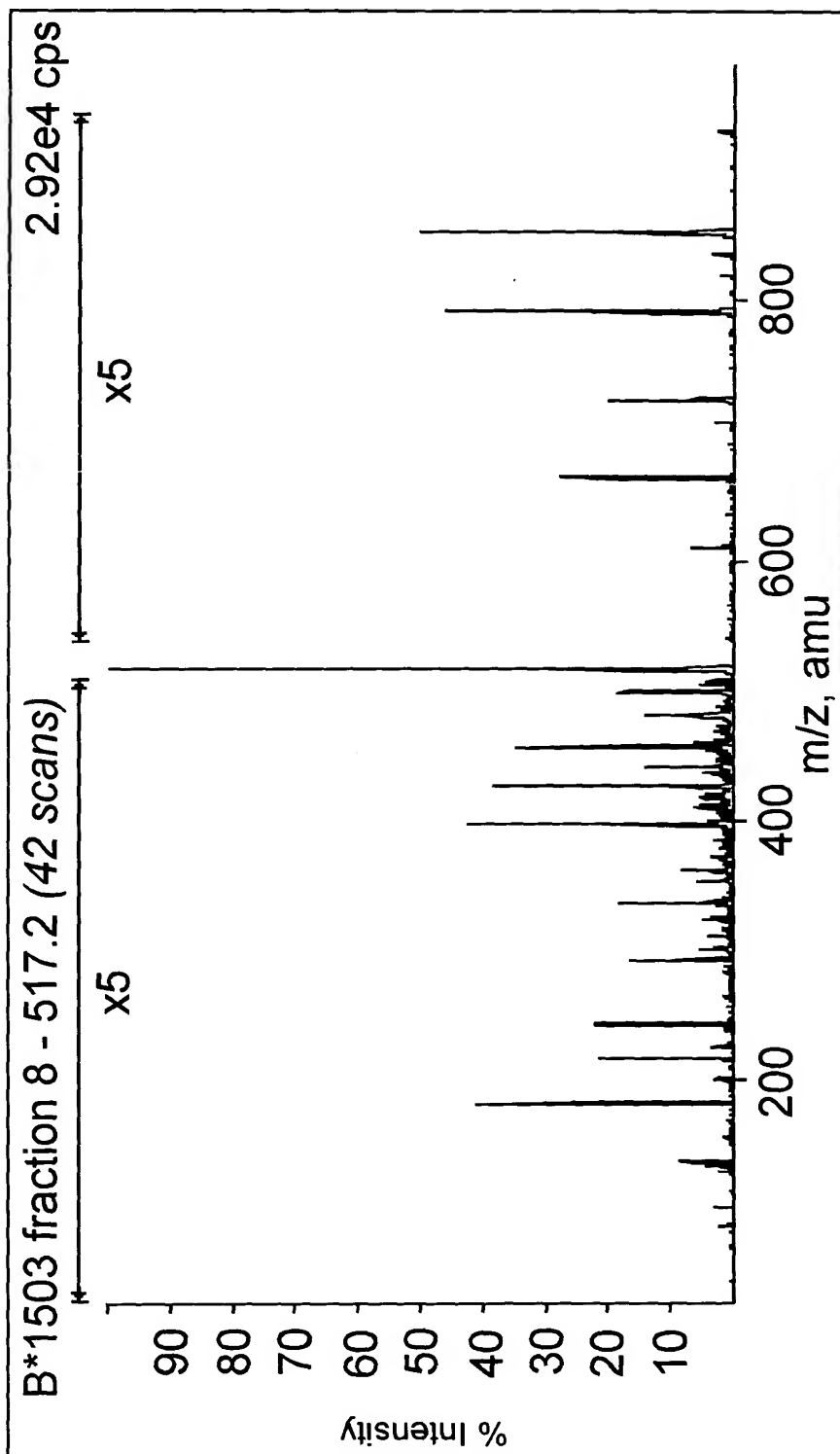


Fig. 3 2 of 3

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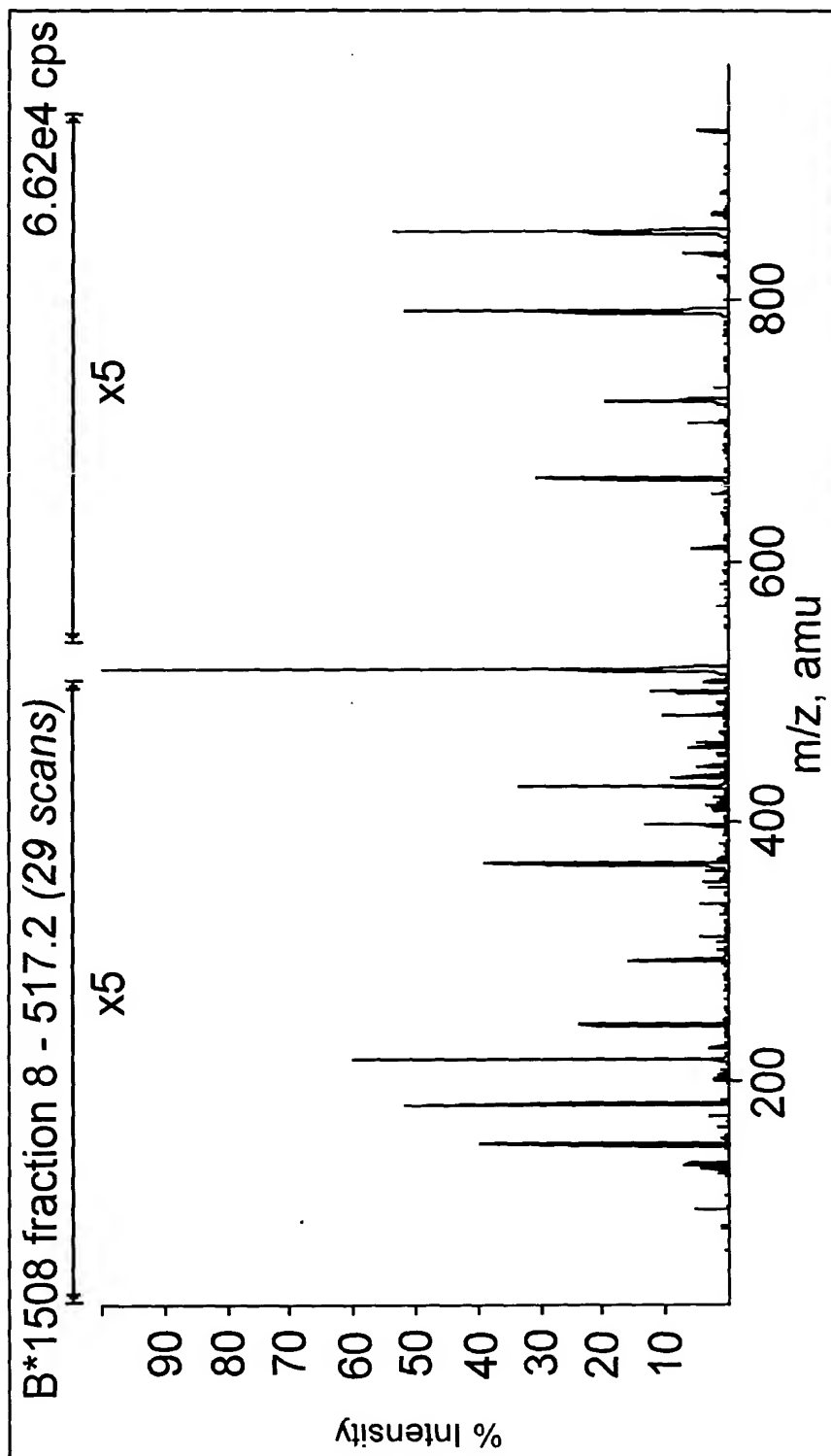
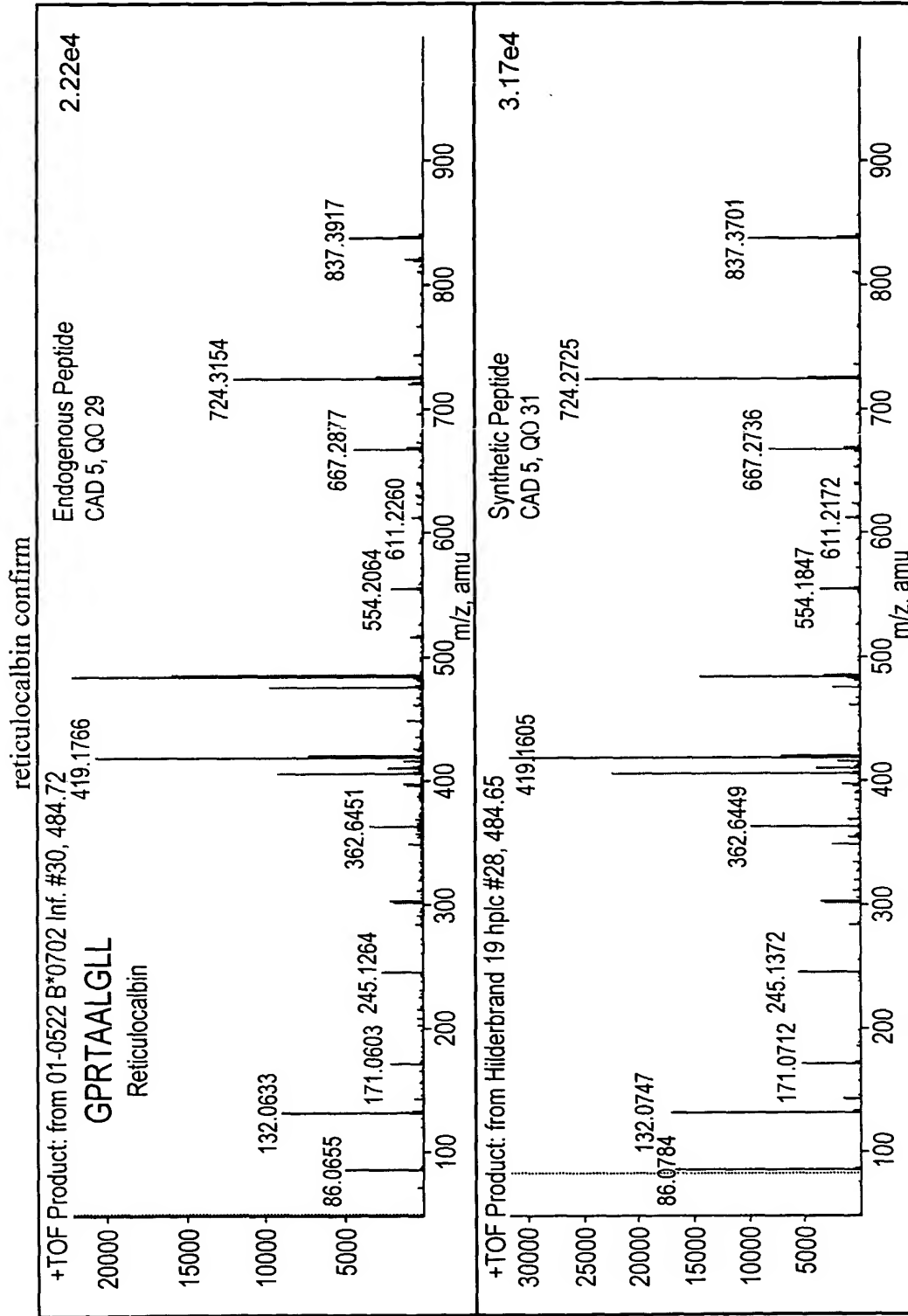


Fig. 3 3 of 3



sHLA B\*0702 was secreted from HIV infected and uninfected cells. The ion maps of the peptides eluted from sHLA B\*0702 in infected and uninfected cells were compared. Ion 484.72 was unique to the HIV infected cells. Ion 484.72 was subjected to MS/MS fragmentation-sequencing. We called GPRTAALGLL as the sequence of the ligand. We synthesized this peptide and found that it generated the same MS/MS fragmentation pattern as the ligand from HIV infected cells. This MS/MS data on a synthetic ligand matches our experimental data and validates the accuracy of our sequence.

Fig. 4

209230 "HEADBOOT"

Serial No. 10/082,034 Dkt. No. 6680.040  
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 Applicant: William Hildebrand Group No.: 2171  
 Filed: 02/21/2002 Examiner: Unknown  
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B\*1508

1	2	3	4	5	6	7	8	9
-	P	N	-	-	-	-	-	Y
-	A	F	K	R	H	Y	I	
-	-	-	E	H	R	-	-	F
-	-	-	D	G	S			

*dominant*

*strong*

B\*1501

1	2	3	4	5	6	7	8	9
-	Q	K	-	-	-	-	-	Y
-	F	N	R	Y				
-	M	P	P	G	-	-	-	F
-	L	H	D	I				
-	V	A	G	E				

*dominant*

*strong*

B\*1510

1	2	3	4	5	6	7	8	9
-	H	Y	P	-	-	-	-	L
-	A	I	K	S	F	L	V	T
-	-	-	G	R	R	I	M	-
-	-	-	D	D	V	L		
-	-	-	E	K				
-	-	-	M	E				
-	-	-	N					

*dominant*

*strong*

Fig. 5

**FBI**

QOYAAAGESFY	ILGPFGSVY	--WDRHTXF	FLZAMGSTY	YMTV---F	SVSXPHAP
TPHTZHEY	XLGDNMY	-----YT	GOYVVZPTY	APAV---VGY	APFTGGNGX
YM---FMY	AP---XVSY	ALGA--RGY	PMFDPZPTF	-----TGF	EHVASSPAL
MVGXXPAT	YMSGXYGTF	XS---VEY	XAVGHSGTY	PVPNVXNY	HHAPCGVSV
DPHYVSGHZF	---AVVAZSY	AQFASGAGZ	-----PTY	-----TXSX	NHAIIVSTSV
VVACV---Y	MPAGYNNVY	-G--CDY	-----PSY	YMVCAEEY	GHNZSVTSTV
PLA-N-HTY	XPVVPAAZTY	---ZRGY	EPAMVXZCF	-----XRDXY	SHZAPCTSV
VVAPITTYG	YMIDPSGVSY	ALNGRVMTY	XAHTCEPRGY	SLX-----F	EHVARSX
XAXYRMXY		DZAPVZMTAY	VQGPVGZTY	ALGSZAXMPF	HHSDGSVSL
PXAMQXYTY		NQZHGSAAEY	TGAPVSEEGY	VGYVDDTOF	MCZ-GMPAX
-P-MPGXAY		FGXACXATSY	VQXYGVSUV	DVEGWMSZY	GHGANDPAPX
---TZNAY		APMARGZY	GPGAPXGGZY	AQHPXAXRF	XHSZPAGPAX
MAAMVGAVAY		TPPTRRRESY	GLGZTSAEF	-----ZAY	XHVVS--VX
LPHQPLATY		GOZZAVDF	AAHWHVEAY	NAXG--RESSF	YHGSZNPZEX
FVTXNXEEY		TPXGEPYZSY	FPTDRRSZF	TARVXSVEY	EHGXENGH
GPZVMXZHY		GQHASVXSX	YTGVSYXHF	AAFCG---XV	AHZAAPPPTX
FOARXTEY		FVSNHAY	AQASAPDAY	XLH--ET	FTACZNPAX
AAAXV---VTY		-----SY	GQRKGAGSVF	ILGPPGSVY	SHAGAGXVX
XPEMGZFSY		NPPAZZPN	VOYYXPF	XLGDNMY	GHXGEPXX
YV--VR-VF		-Q-DPPDMZY	ATGTAZNXNZY	VMGXTNANF	XHGDDHVX
AAPVGAXESY		XQ--AGGSY	VVACV---Y	AVVTXZSDF	YHHDXXVX
YVA---PAF		SQFGGSQY	PLA-N-HTY	AMNPTNTVF	MAGAWCRX
VGY--AHPGF		SQFDHVTY	VVAPITTY	SQXAAAGVDVF	FH---XX
-----STY		---AAHVPPGY	QOYTVGYF	-VFSHTTF	EH---TVX
SPTYTHAVAF		FMDVGAPTVY	PLFGZTAGZY	XQGHHEMFY	MAX-----VX
MFA--MVMAF		XAN---SEY	VAGGW---F	HLTGTNEATSF	-----PVX
XA---SYTY		AQM---SEY	CPLSCFT	SGAXDRAYZF	XHYDRNZX
VGYVDDTOF		ZATNSVSTSY	YATAGEMMAF	YMGIDPSGVSY	-----AXSV
YATAGEMMAF		YATAGEMMAF	FLZAMZSTY	VOGPVGTDF	XHWPVNEX
TARVXSVEY		-P---PSSGY	TVXDSZTHY	FQARXTEY	-H-----PVF
MPAADYEVAF		MPAADYEVAF	AQAAPFAGY	XAGFFXXEY	XHEVZPXHX
AAFCG---XV		AAFCG---XV	XQ-----ZY	XQ-----ZY	-HGCPGMPX
SPNEDXMZVF		SPNEDXMZVF	FQ-----TX	FQ-----TX	ETPEHAPVX
VAATAGAVE		VAATAGAVE	ALW--PZF	TP---AZAF	MXPGNSAXXY
XLH--ET		XLH--ET	VPHZNAY	-----GHGGY	
YVS--RNZY		YVS--RNZY	VVATZNZZX		

Fig. 5 continued



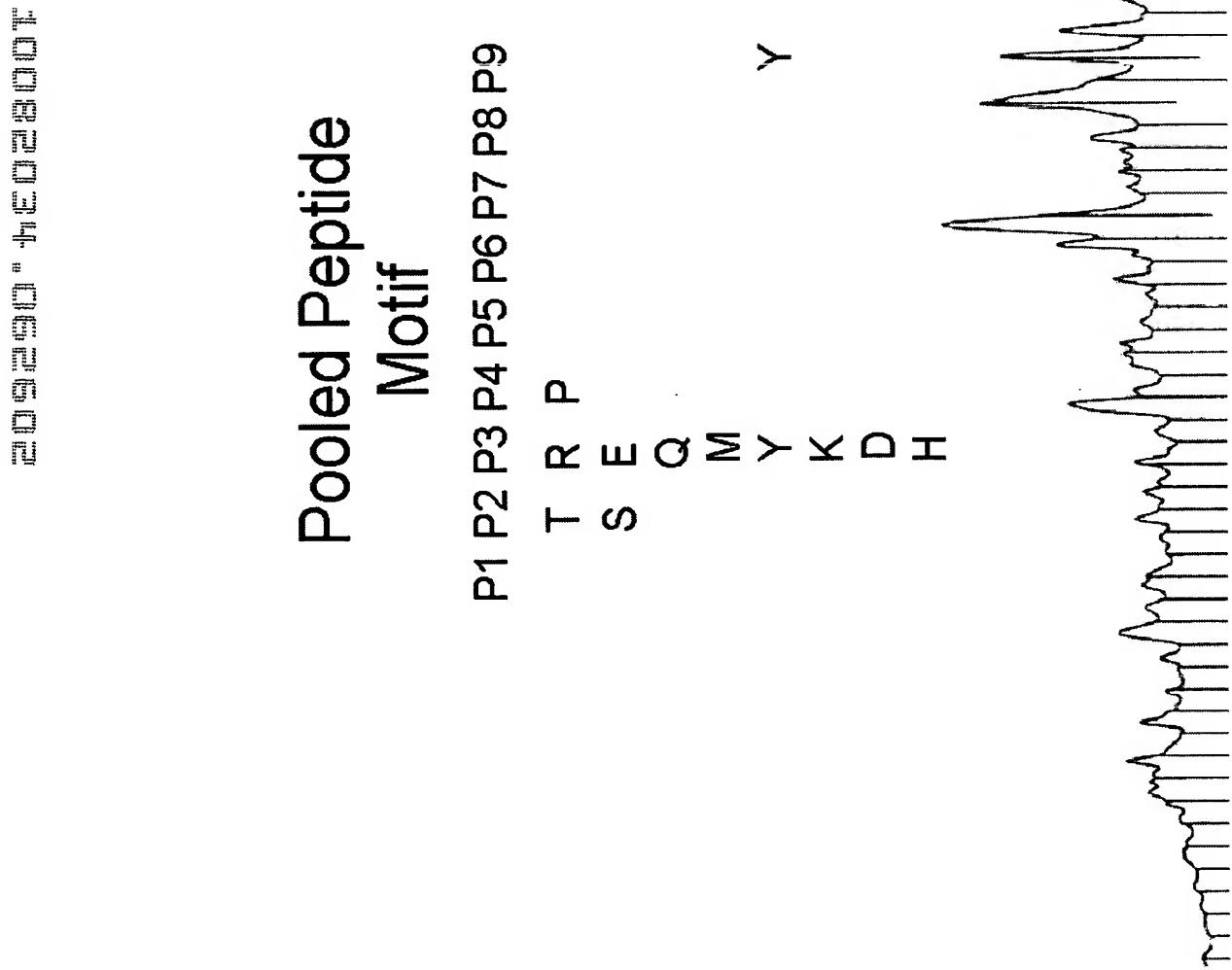


Fig. 6

2092910\*HE02803T

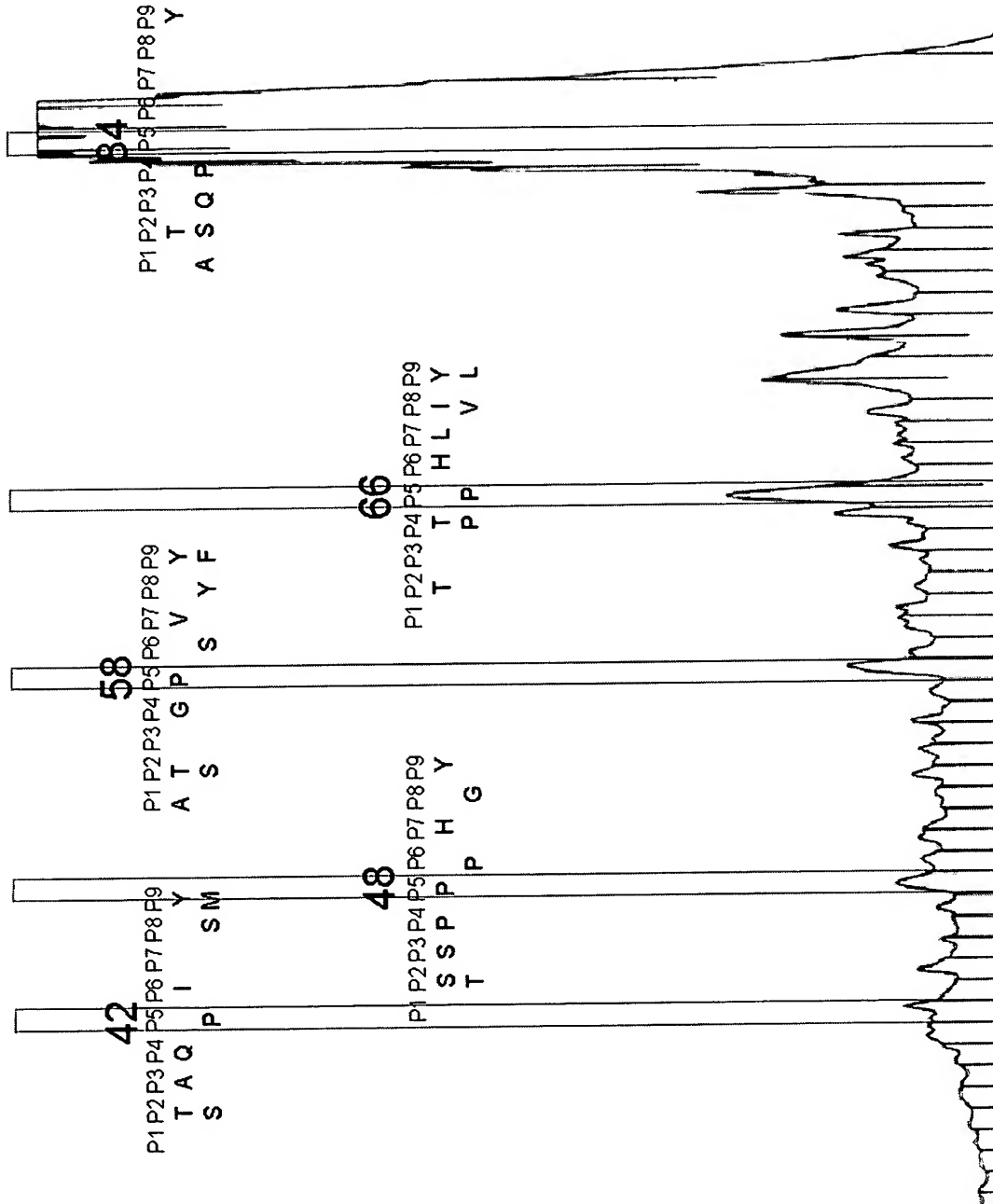


Fig. 7

209230"4E02800T

Narrowing search parameters using fraction motifs:

Ovarian Carcinoma Immunoreactive Antigen				
MNGRADFREP	NAEVPRIPIH	IGPDYIPTEE	ERRYFAECND	ESFWFRSYPL
AATSMILTQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSTVVKTCQE
KFKKLENSPL	GEALRSGQAR	RSSPPGHYYQ	KSKYDSSVSG	QSSFVTSPAA
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SAPTGITDHI	YQGPDPNLEE
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVNIKYG
DTWDE				

Scanning with whole-pooled motif revealed 4 putative epitopes.

Ovarian Carcinoma Immunoreactive Antigen				
MNGRADFREP	NAEVPRIPIH	IGPDYIPTEE	ERRYFAECND	ESFWFRSYPL
AATSMILTQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSTVVKTCQE
KFKKLENSPL	GEALRSGQAR	RSSPPGHYYQ	KSKYDSSVSG	QSSFVTSPAA
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SAPTGITDHI	YQGPDPNLEE
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVNIKYG
DTWDE				

Scanning with fraction 48 peptide motif revealed 1 putative epitope.

Fig. 8

209230-1E0280.DT

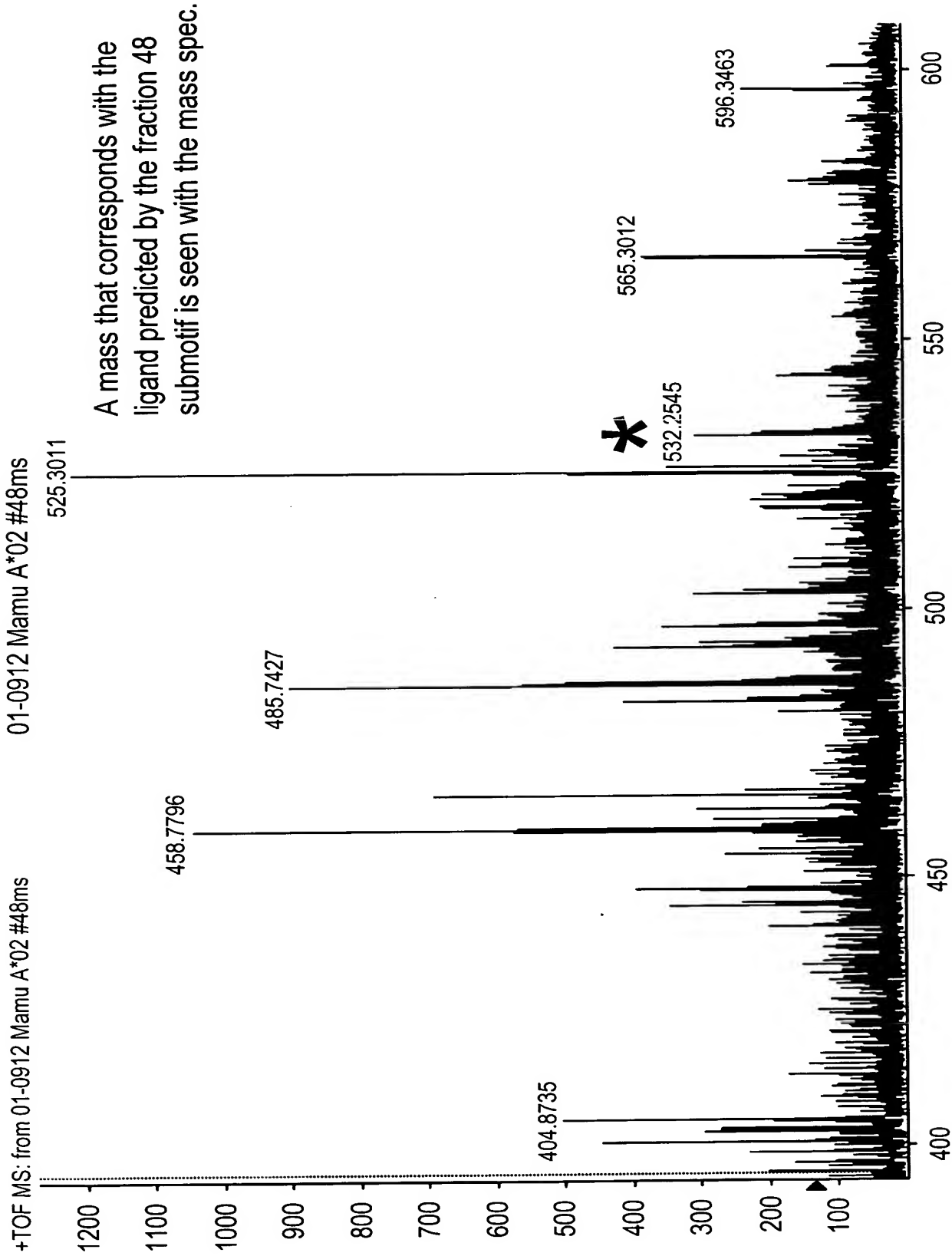


Fig. 9  
m/z, amu

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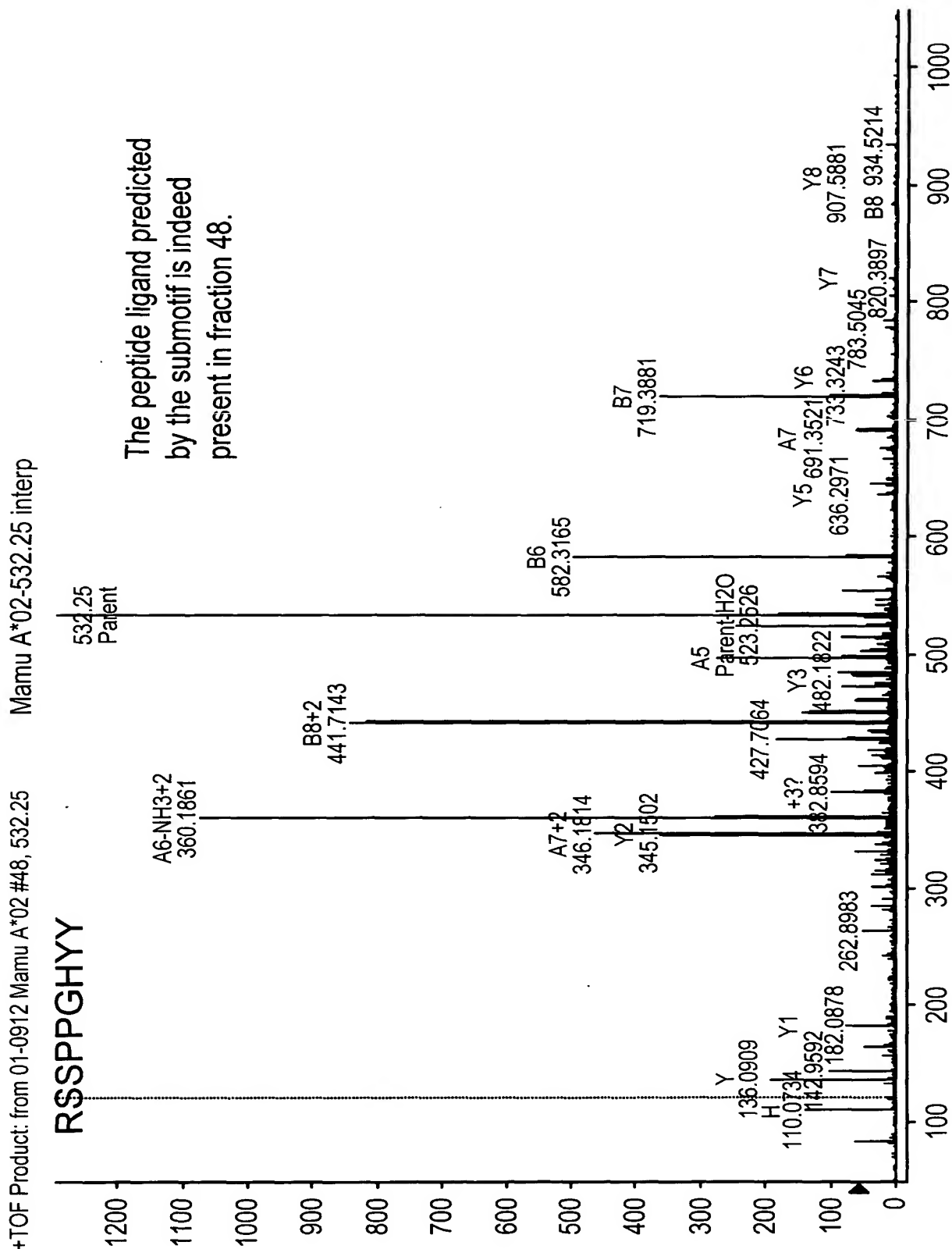


Fig. 10 m/z, amu

Motif Data (Edman sequencing)

	1	2	3	4	5	6	7	8	9
	%	fold	%	fold	%	fold	%	fold	%
Dominant 3.5 fold increases or more over prior round			F 9.20						
			L 7.60						
			N 6.20						
			M 4.90						
Strong 2.5-3.5 fold increase over prior round									
	K 31.50	R 53.80	Q 8.20	P 8.30					L 7.50
	R 15.50		K 5.40						
	S 10.40		L 5.10						
Weak 2.0-2.5 fold increase over prior round									
			A 5.80		M 4.90		T 7.70		
Trace 1.50-2.0 fold increase over prior round									
		Q 3.60	R 2.20	K 11.00	I 6.50			Q 11.40	
				S 6.00	F 4.20			N 5.70	
				V 5.30	H 1.80				

Fig. 11

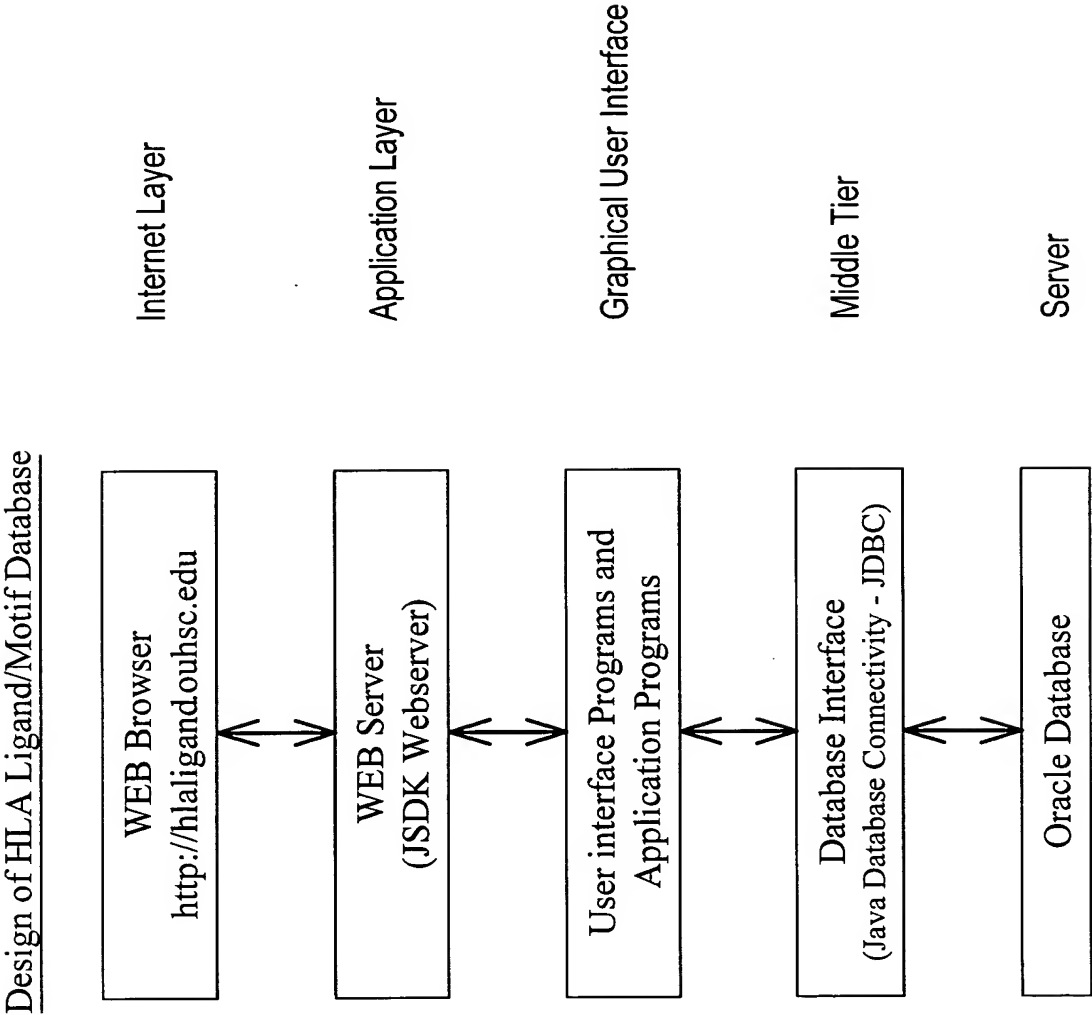


Fig. 12

Entity-Relationship (ER) Diagram for HLA Ligand/Motif Database

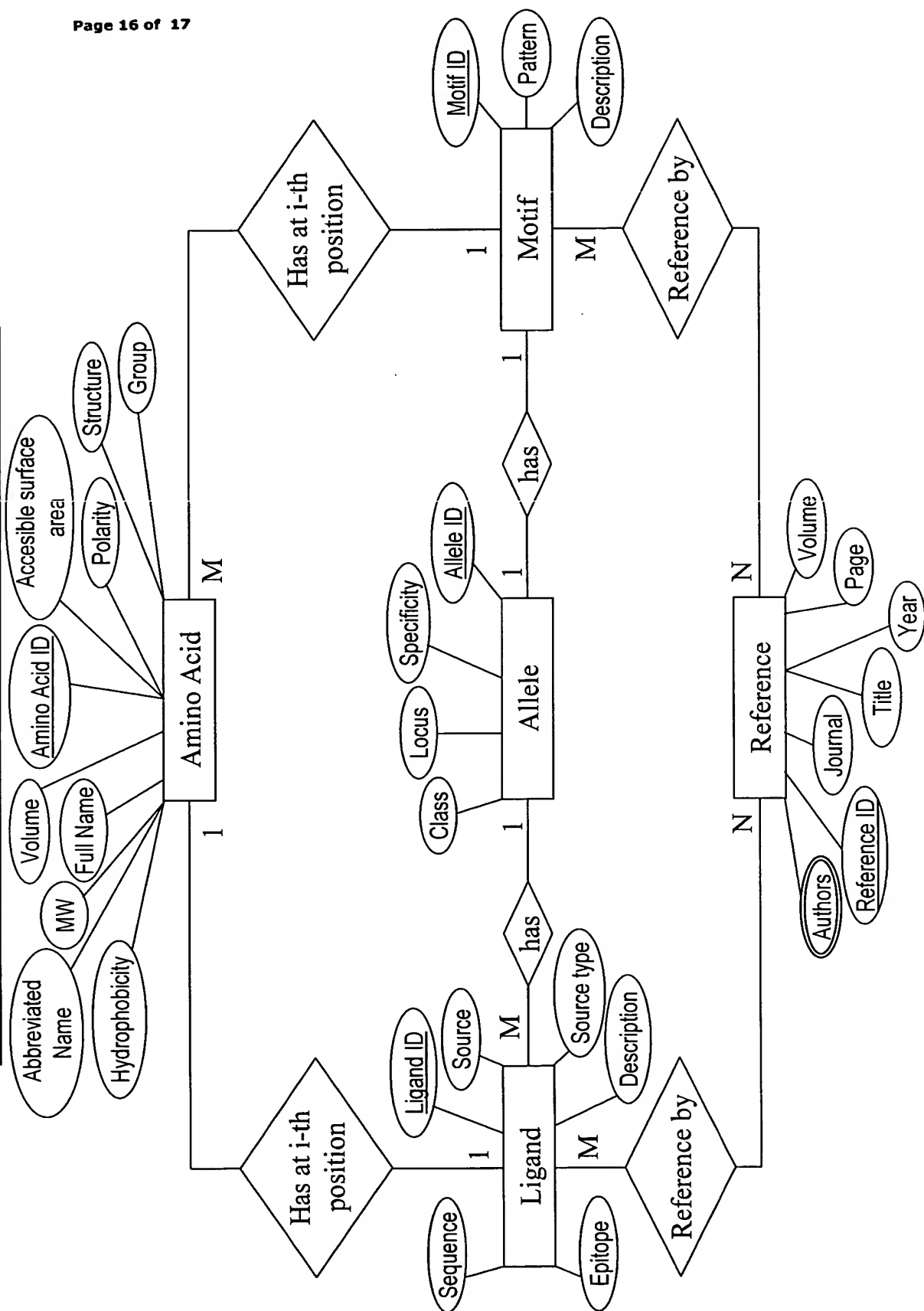


Fig. 13



UML Diagram for HLA Ligand/Motif Database

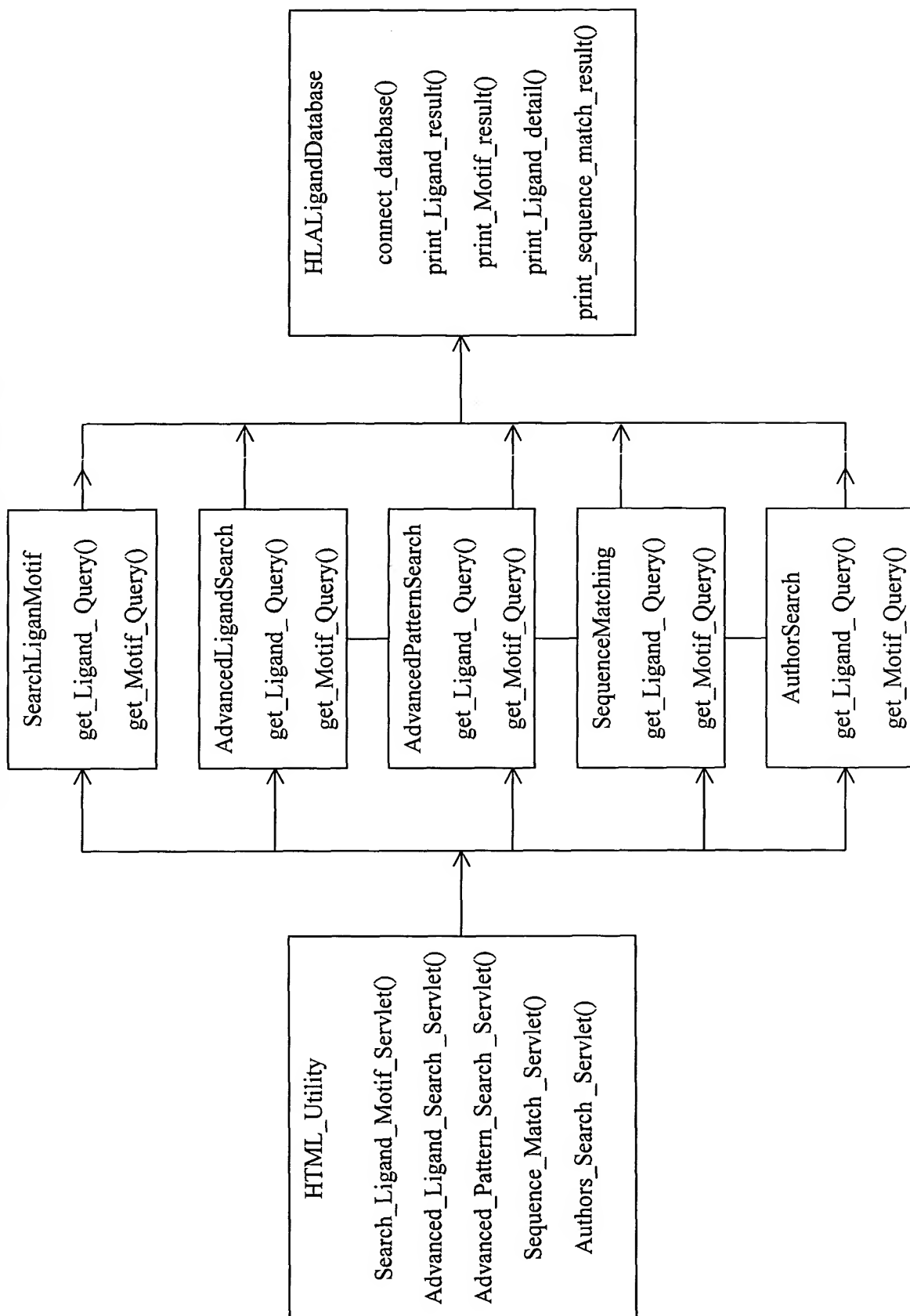


Fig. 14